Understanding HDL function

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CHAPTER 7

Pancreatic β-cell function relates positively to HDL functionality in well-controlled type 2 diabetes mellitus

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Abstract

Background. High-density lipoproteins (HDLs) have been implicated in glucose homeostasis. Among subjects with normal fasting glucose (NFG), impaired fasting glucose (IFG) and type 2 diabetes mellitus (T2DM) we tested whether pancreatic β-cell function relates to HDL functionality, as determined by HDL anti-oxidative capacity and cellular cholesterol efflux to plasma.

Methods and Results. HDL anti-oxidative capacity (inhibition of LDL oxidation *in vitro*), cellular cholesterol efflux (the ability of plasma to stimulate cholesterol efflux out of cultured fibroblasts obtained from a single human donor), glucose and insulin were determined in fasting plasma samples from 37 subjects with NFG, 36 with IFG, and 22 with T2DM (no glucose-lowering drug or insulin treatment; HbA1c 6.0 ± 1.0%). Homeostasis model assessment was used to estimate pancreatic β-cell function (HOMA-β) and insulin resistance (HOMAir). HOMA-β was lowest, whereas HOMAir was highest in T2DM ($P < 0.01$ and $P < 0.001$ vs. NFG). HDL anti-oxidative capacity and cellular cholesterol efflux did not differ significantly according to glucose tolerance category. In univariate analysis and after controlling for HOMAir both HDL anti-oxidative capacity ($P < 0.05$) and cellular cholesterol efflux ($P < 0.01$) were positively correlated with HOMA-β in T2DM, but not in NFG and IFG. In age-, sex- and HOMAir-adjusted analyses, T2DM status interacted positively with HDL anti-oxidative capacity ($P = 0.001$) and cellular cholesterol efflux ($P = 0.042$) on HOMA-β. HbA1c interacted similarly with HDL functionality measures on HOMA-β.

Conclusion. Pancreatic β-cell function relates to pathophysiologically relevant measures of HDL function in T2DM, but not in NFG and IFG. Better HDL functionality may contribute to maintenance of β-cell function in subjects with well-controlled T2DM.

7.1 Introduction

A low level of high-density lipoprotein (HDL) cholesterol represents a well-established determinant of cardiovascular risk.[1] The cardiovascular protection attributed to HDL is at least in part conveyed by the anti-oxidative and anti-inflammatory properties of this lipoprotein fraction.[2] In addition, HDL is able to remove excess cholesterol from peripheral cells, thereby beneficially stimulating the transport of cholesterol from vascular tissues to the liver for metabolism and subsequent biliary excretion.[3]

More recently, HDL has also been proposed to play a pathogenetic role in glucose homeostasis.[4, 5] In analogy with the impact of impaired HDL functionality on increased atherosclerosis susceptibility,[2, 3] it has been hypothesized that abnormalities in HDL's functional properties may result in diminished protection of pancreatic β-cells against oxidative stress, apoptosis, islet inflammation, and cholesterol accumulation.[4] In this vein, *in vitro* studies have demonstrated that HDL protects pancreatic β-cells against apoptosis and may restore oxidized low density lipoprotein (LDL)-induced impairment of insulin processing.[6, 7] In addition, free apolipoprotein (apo) A-I, reconstituted HDL particles, as well as native HDL are able to stimulate insulin secretion by β-cells.[8] Moreover, the insulin secretion pathway is impaired in distinct knock-out models of either the ATP-binding transporter cassette A1 (ABCA1) [9, 10] or ABCG1,[11] transporters that play a critical role in the removal of cholesterol from cells to the extracellular space.[12] In line, humans with heterozygous ABCA1 deficiency show a reduced insulin response to intravenous glucose.[13] Of further interest, short-term administration of reconstituted HDL increases plasma insulin and lowers glucose in subjects with type 2 diabetes mellitus (T2DM).[14]

In keeping with the notion that HDL may exert beneficial effects on glucose homeostasis, several epidemiological studies have shown that lower levels of HDL cholesterol confer a higher incidence of T2DM independently of alterations in apoB-containing lipoproteins and obesity.[15, 16] Remarkably little is currently known about the relationship of pancreatic β-cell function with HDL functionality in humans.

The present study was initiated to test whether pancreatic β-cell function relates to HDL functional properties in T2DM, and whether such a relationship is different in T2DM subjects compared to subjects with normal fasting glucose (NFG) and impaired fasting glucose (IFG). For this purpose, we used two distinct read-outs for HDL function, represented by the capacity to inhibit LDL oxidation *in vitro* [17, 18] and the ability of plasma to stimulate cholesterol efflux out of cultured fibroblasts obtained from a single human donor.[19]

7.2 Materials and Methods

7.2.1 Subjects

The medical ethics committee of the University Medical Center Groningen approved the study protocol. The study was carried out in a University Hospital setting. Study participants with and without T2DM were aged > 18 years, and were recruited by advertisement in local newspapers. Written informed consent was obtained from all participants. T2DM had been previously diagnosed by primary care physicians using World Health Organization (WHO) criteria.[20] T2DM patients using insulin or oral glucose-lowering drugs were excluded to obviate effects on β-cell function and insulin sensitivity

estimates.[21] They were treated with diet and received lifestyle advice only. The use of antihypertensive medication was allowed. In non-diabetic subjects, glucose tolerance status was classified as NFG (plasma glucose < 6.1 mmol/l) or IFG (plasma glucose ≥ 6.1 and ≤ 6.9 mmol/l), using WHO cut-off criteria.[20] Subjects who used lipid-lowering drugs were excluded in order to minimize possible confounding on HDL variables. Current smokers, subjects with a history of cardiovascular disease, chronic kidney disease, liver function abnormalities or thyroid dysfunction were also excluded. Maximal alcohol intake was 3 beverages per day.

To replicate relationships of HDL cholesterol and apoA-I with β-cell function and insulin sensitivity, correlation analyses were also carried out in another group of non-diabetic men (213 subjects with NFG and 12 subjects with IFG), representing a sub-cohort from the Prevention of Renal and Vascular End-stage Disease (PREVEND) population (www.PREVEND.org).

Homeostasis model assessment was used to estimate β-cell function (HOMA-β) and insulin sensitivity (HOMAir).[22] HOMA-β was calculated using the equation: 20 × fasting insulin (mU/l)/[fasting glucose (mmol/l) − 3.5]. HOMA-β represents the relative β-cell function of an individual and is expressed as a percentage.[22] HOMAir was calculated with the equation: fasting plasma insulin (mU/l) × fasting plasma glucose (mmol/l)/22.5.

All participants were evaluated after an overnight fast. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Blood pressure was measured after 15 min rest at the left arm in sitting position using a sphygmomanometer.

7.2.2 Laboratory methods

Venous blood samples were collected into ethylene diamine tetraacetic acid (EDTA)-containing tubes (1.5 mg/ml). Plasma samples were stored at −80˚C until analysis. Plasma glucose and glycated hemoglobin (HbA1c) were measured shortly after blood collection. Plasma cholesterol and triglycerides were assayed by enzymatic methods (Roche/Hitachi cat. no. 11876023 and 11875540 respectively, Roche Diagnostics GmbH, Mannheim, Germany). HDL cholesterol was measured using a homogeneous enzymatic colorimetric test (Roche/Hitachi cat. no. 03030024). ApoA-I was determined by immunoturbidimetry (Roche/Cobas Integra Tina-quant cat. no. 03032566, Roche Diagnostics).

HDL anti-oxidative capacity was determined as described.[17, 18] To this end apoB-containing lipoproteins were precipitated by adding 75 µl polyethylene glycol-6000 in 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) at pH 8.0 to 150 µl of plasma followed by mixing and an incubation on ice for 30 min. Subsequently, samples were centrifuged at 2000 × *g* at 4°C for 30 min. Supernatants were transferred to clean tubes and the HDL cholesterol concentration was determined using a commercially available kit (Roche Diagnostics GmbH, Mannheim, Germany). Native LDL was isolated from a normolipidemic donor by density gradient ultracentrifugation (1.019 < *d* < 1.063 g/l). All samples were processed and assayed on the same day using identical reagents. Individual HDL preparations (0.26 mmol/l cholesterol), isolated as detailed above, were added to aliquots of native LDL (1.95 mmol/cholesterol) followed by the addition of 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH, Cayman Chemicals, Ann Arbor, MI, USA) in a final concentration of 1 mM in a total volume of 110 µl. Samples were then incubated for 24 h at 37°C to induce LDL oxidation. Thiobarbituric acid reactive substances (TBARS) were determined as a measure for the degree of LDL oxidation.[23] To

this end 200 µl 10% trichloroacetic acid was added to 100 µl sample and incubated on ice for 15 min. Subsequently, the mixtures were centrifuged at 2200 × *g* at 4°C for 15 min. Thereafter, 200 µl of the supernatant was transferred to new reaction tubes and 200 µl 0.67% thiobarbituric acid was added to each sample. These mixtures were incubated in a boiling waterbath for 10 min and chilled on ice. Finally, fluorescence was measured and the amounts of TBARS in the samples were calculated using a standard curve. Native LDL before incubation contained no measurable amounts of TBARS and TBARS present in individual HDL samples were also negligible compared with the increase caused by AAPH addition. Therefore, TBARS formation in native LDL samples subjected to the same procedures but without the addition of AAPH was used as blanks. The HDL anti-oxidative capacity was calculated as the difference between the maximal amount of TBARS formed in a reaction to which no HDL had been added, and the percent reduction that was obtained with an individual HDL sample. Thus, higher values indicate better protection against oxidation. The inter-assay coefficient of variation amounts to 5.1%.

Cholesterol efflux to whole plasma was assayed using human fibroblasts as cholesterol donor as described.[19] Fibroblasts were obtained from a normolipidemic non-diabetic subject by explant culture from a 3 mm punch biopsy at a 1 mm skin thickness and were cultured (until passage 5–15) in 24 wells culture plates to full confluence. The cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) fetal calf serum (FCS). After washing with DMEM, the cells were loaded with [^{3}H]-cholesterol (0.5 µCi m/l) during 24 h in the presence of added unlabelled cholesterol (30 µg/ml) in order to induce ABCA1 in the fibroblasts.[19] [^{3}H]-cholesterol and unlabelled cholesterol were solubilized in ethanol and diluted into the efflux medium. After cholesterol loading, the cells were washed 3 times with phosphate buffered saline (PBS)/bovine serum albumin (BSA) 0.2% (w/v). The efflux assay was started by adding the plasma diluted to 1% in efflux medium. 1.25 U/ml heparin was added to prevent clotting. The addition of heparin does not influence cholesterol efflux rates.[19] After 4 h incubation at 37°C the medium was collected and centrifuged. Thereafter, [^{3}H]-cholesterol was quantified by liquid scintillation counting. Total cellular [^{3}H]-cholesterol was determined after extraction of the cells with 2-propanol. The percentage efflux was calculated by dividing the radioactive counts in the efflux medium by the sum of the counts in the medium and the cell extract. The samples were analyzed in duplicate and values were corrected for radioactivity appearing in the culture medium in the absence of plasma. To be able to normalize results between series of experiments and to correct for day-to-day variation, efflux to a human plasma pool was determined in quadruplicate. Since this assay system estimates outward flux of labelled cholesterol from the fibroblasts, the influence of back flux of cholesterol from plasma into the cells was evaluated using double isotope labeling experiments.[19] These experiments indicated that under the present assay conditions transport of cholesterol was almost exclusively from cells to the medium.[19] The inter-assay coefficient of variation is 6%.

Plasma glucose was measured with a glucose analyzer (APEC Inc., Danvers, MA, USA). Plasma insulin was measured with a microparticle enzyme immunoassay (AxSYM insulin assay; Abbott Laboratories, Abbott Park IL, USA). HbA1c was measured by high performance liquid chromatography (Bio-Rad, Veenendaal, The Netherlands; normal range 4.6–6.1%).

7.2.3 Statistical analysis

SPSS 18 was used for data analysis. Results are expressed in mean ± SD or in median (interquartile range). Because of skewed distribution logarithmically transformed values of HOMA-β and HOMAir were used. Differences in continuous variables according to glucose tolerance status category were assessed by one-way analysis of variance (ANOVA) with subsequent Bonferroni correction for multiple comparisons. Differences in proportions between the glucose tolerance groups were assessed by χ^2-analysis. Univariate correlation coefficients were calculated using linear regression analysis. In view of the (mathematical) interdependence of HOMA-β and HOMAir,[21, 22] partial correlations of HOMA-β with HDL cholesterol, apoA-I and HDL functionality measures were calculated after controlling for HOMAir. Multiple linear regression analyses were carried out to determine the independent contributions of HDL functionality measures to HOMA-β (HOMA-β being the dependent variable). All multiple linear regression analyses were adjusted for age, sex, and HOMAir. Interaction terms were calculated as the product terms between glucose tolerance categories (using dummy variables with NFG as the reference category), and in alternative analyses between the HbA1c level and the HDL functionality measure of interest (anti-oxidative capacity and cellular cholesterol efflux). In these models, glucose tolerance categories, or alternatively the HbA1c level, and the HDL functionality measure of interest were included together with the interaction terms. For continuous variables (HDL functionality measures and HbA1c) distributions centered to the mean were made by subtracting the individual value of the variable of interest from their respective group mean values to account for possible outliers. Interaction terms were considered to be statistically significant at two-sided *P* values < 0.10.[24] Otherwise, the level of significance was set at two-sided *P* values < 0.05.

7.3 Results

Thirty-seven subjects with NFG, 36 subjects with IFG, and 22 subjects with T2DM participated in the study (Table 1). Twenty-nine of the 49 women participating in the study were post-menopausal. Estrogens were used in 2 pre- and in 2 post-menopausal women (NFG, n = 2; IFG, n = 1; T2DM, n = 1). Among T2DM subjects diabetes duration was 4 (interquartile range 2.6–5.1) years. Antihypertensive drugs were used in 5 T2DM subjects (angiotensin converting enzyme inhibitors, n = 2; beta-blockers, n = 2; diuretics, n = 1). Other medications were not used.

As shown in Table 7.1 NFG subjects were younger compared to the other groups. Sex distribution, BMI, systolic and diastolic blood pressure, and plasma insulin levels were not significantly different between the glucose tolerance groups. HOMA-β was higher in NFG subjects compared to IFG subjects and T2DM subjects, whereas HOMAir was decreased in T2DM subjects compared to NFG and IFG subjects (Table 7.1). In addition, the HOMA-β/HOMAir ratio was found to be lower in IFG vs. NFG subjects (*P* < 0.001) and in T2DM vs. IFG subjects (*P* < 0.001). HbA1c was higher in T2DM compared to the other groups. Plasma total cholesterol, triglycerides, HDL cholesterol, and apoA-I levels were not different between the groups. HDL anti-oxidative capacity and cellular cholesterol efflux were also not significantly different between the groups (Table 7.1).

Table 7.1. Clinical characteristics, β-cell function (HOMA-β), insulin sensitivity (HOMAir), plasma lipids, apolipoprotein A-I, HDL anti-oxidative capacity, and cellular cholesterol efflux to plasma according to glucose tolerance (normal fasting glucose, n = 37; impaired fasting glucose, n = 36; type 2 diabetes mellitus, n = 22).

	Normal fasting glucose (n = 37)	**Impaired fasting glucose (n = 36)**	**Type 2 diabetes mellitus (n = 22)**	***P* value**
Age (years)	52 ± 9[a,d]	57 ± 10	61 ± 9	<0.001
Sex (men/women)	13/24	21/15	12/10	0.20
BMI (kg m^2)	25.9 ± 4.3	25.5 ± 3.2	26.8 ± 4.9	0.50
Systolic blood pressure (mm Hg)	129 ± 20	132 ± 20	139 ± 18	0.16
Diastolic blood pressure (mm Hg)	83 ± 13	81 ± 9	81 ± 6	0.67
Plasma glucose (mmol/l)	5.1 ± 0.4[c,e]	6.1 ± 0.3[c]	8.0 ± 1.9	<0.001
Insulin (mU/l)	5.9 (4.5-8.6)	7.1 (4.7-8.6)	8.7 (5.4-13.3)	0.16
HOMA-β (%)	106.4 (48.9-122.8)[b,d]	51.8 (37.4-72.4)	36.7 (24.7-72.3)	<0.001
HOMAir ((mU mmol/l^2) /22.5)	1.41 (1.00-2.01)[c]	1.89 (1.25-2.55)[b]	2.82 (1.91-3.92)	<0.001
HOMA-β/HOMAir	55.17 (43.86-60.00)[c,f]	28.37 (24.56-33.73)[c]	13.42 (9.23-25.85)	<0.001
HbA1c (%)	5.3 ± 0.4[c]	5.3 ± 0.5[c]	6.0 ± 1.0	<0.001
Total cholesterol (mmol/l)	5.66 ± 1.02	5.71 ± 0.87	5.38 ± 1.28	0.48
Triglycerides (mmol/l)	1.16 (0.82-1.82)	1.34 (0.97-1.96)	1.60 (0.90-2.20)	0.62
HDL cholesterol (mmol/l)	1.49 ± 0.39	1.50 ± 0.42	1.48 ± 0.45	0.98
Apolipoprotein A-I (g/l)	1.40 ± 0.21	1.45 ± 0.23	1.45 ± 0.23	0.51
HDL anti-oxidative capacity (%)	62.4 ± 11.7	66.9 ± 10.4	62.4 ± 10.1	0.15
Cellular cholesterol efflux to plasma (% per 4h)	8.4 ± 1.1	8.7 ± 0.9	8.5 ± 1.0	0.56

Data in mean ± SD or median (interquartile range). BMI, body mass index; HDL, high-density lipoproteins.
[a] $P < 0.05$ vs. subjects with type 2 diabetes mellitus; [b] $P < 0.01$ vs. subjects with type 2 diabetes mellitus; [c] $P < 0.001$ vs. subjects with type 2 diabetes mellitus; [d] $P < 0.05$ vs. subjects with impaired fasting glucose; [e] $P < 0.01$ vs. subjects with impaired fasting glucose; [f] $P < 0.001$ vs. subjects with impaired fasting glucose.

In univariate analysis HDL cholesterol and plasma apoA-I levels were correlated inversely with fasting plasma insulin, HOMA-β and HOMAir in NFG and IFG subjects, but not significantly so in T2DM subjects (Table 7.2). In the combined subjects, inverse univariate relationships of HDL cholesterol and apoA-I with HOMA-β and with HOMAir were also present. HDL anti-oxidative capacity and cellular cholesterol efflux to plasma were not significantly correlated with plasma insulin, HOMA-β, and HOMAir in NFG and IFG subjects. In contrast, HOMA-β was correlated positively with HDL anti-oxidative capacity and with cellular cholesterol efflux to plasma in T2DM subjects (Table 7.2). In T2DM subjects, HDL anti-oxidative capacity was also correlated inversely with plasma glucose, whereas

cellular cholesterol efflux was correlated positively with plasma insulin and HOMAir. HOMA-β and HOMAir were unrelated to diabetes duration ($P > 0.10$, data not shown). Furthermore, the relationships of HDL anti-oxidative capacity and of cellular cholesterol efflux to plasma with HDL cholesterol and with apoA-I did not reach significance in the 3 groups ($P > 0.05$, data not shown).

Table 7.2. Univariate correlations of plasma glucose, insulin, β-cell function (HOMA-β), and insulin sensitivity (HOMAir) with HDL cholesterol, plasma apolipoprotein A-I, HDL anti-oxidative capacity, and cellular cholesterol efflux to plasma in subjects with normal fasting glucose, impaired fasting glucose, and type 2 diabetes mellitus.

	Plasma glucose	Plasma insulin	HOMA-β	HOMAir
Normal fasting glucose (n = 37)				
HDL cholesterol	0.053	-0.600***	-0.583***	-0.639***
Apolipoprotein A-I	0.002	-0.528***	-0.486**	-0.575***
HDL anti-oxidative capacity	0.128	0.272	0.144	0.257
Cellular cholesterol efflux	0.027	0.121	0.158	0.198
Impaired fasting glucose (n = 36)				
HDL cholesterol	-0.079	-0.418*	-0.509**	-0.528***
Apolipoprotein A-I	-0.045	-0.334*	-0.425**	-0.431**
HDL anti-oxidative capacity	0.034	-0.176	-0.181	-0.167
Cellular cholesterol efflux	0.027	0.121	0.158	0.198
Type 2 diabetes mellitus (n = 22)				
HDL cholesterol	-0.194	-0.258	-0.169	-0.396
Apolipoprotein A-I	-0.301	-0.018	0.086	-0.169
HDL anti-oxidative capacity	-0.576**	0.184	0.425*	-0.060
Cellular cholesterol efflux	-0.332	0.589**	0.657***	0.464*
Combined subjects (n = 95)				
HDL cholesterol	-0.070	-0.440***	-0.405***	-0.486***
Apolipoprotein A-I	-0.024	-0.308**	-0.326***	-0.336***
HDL anti-oxidative capacity	-0.144	0.093	0.091	0.052
Cellular cholesterol efflux	-0.071	0.184	0.181	0.174

Pearson correlation coefficients are shown. HOMA-β and HOMAir values are logarithmically transformed.
* $P < 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Strong univariate correlations were observed between HOMA-β and HOMAir (NFG, r = 0.880, $P < 0.001$; IFG, r = 0.932, $P < 0.001$; T2DM, r = 0.496, $P = 0.019$; combined subjects r = 0.507, $P < 0.001$). In order to take account of the (mathematical) interdependence between HOMA-β and HOMAir, the relationships of HOMA-β with HDL cholesterol, apoA-I, HDL anti-oxidative capacity, and cellular cholesterol efflux to plasma were also determined after adjustment for HOMAir. In these analyses the positive relationships of HOMA-β with HDL anti-oxidative capacity and cellular cholesterol efflux in T2DM subjects remained significant after controlling for HOMAir (partial correlation coefficients are given in Table 7.3). In NFG and IFG subjects, the relationships of HOMA-β with HDL

cholesterol and apoA-I were not significant when HOMAir was taken into account (Table 7.3).

Table 7.3. Partial correlation coefficients of β-cell function (HOMA-β) with HDL cholesterol, plasma apolipoprotein A-I (apoA-I), HDL anti-oxidative capacity, and cellular cholesterol efflux after controlling for insulin sensitivity (HOMAir) in subjects with normal fasting glucose, impaired fasting glucose, and type 2 diabetes mellitus.

	HDL cholesterol	ApoA-I	HDL anti-oxidative capacity	Cellular cholesterol efflux
Normal fasting glucose (n = 37)				
HOMA-β	-0.056	0.051	-0.177	-0.035
Impaired fasting glucose (n = 36)				
HOMA-β	-0.057	-0.071	-0.069	0.116
Type 2 diabetes mellitus (n = 22)				
HOMA-β	0.034	0.198	0.525*	0.554**

HOMA-β and HOMAir values are logarithmically transformed.
* $P < 0.05$; ** $P \leq 0.01$.

Table 7.4. Multiple linear regression analyses demonstrating interactions between glucose tolerance category (normal fasting glucose, NFG; n = 37, impaired fasting glucose, IFG; n = 36 and type 2 diabetes mellitus, T2DM; n = 22) and HDL anti-oxidative capacity (model A) or cellular cholesterol efflux to plasma (model B) on β-cell function (HOMA-β).

	Model A		Model B	
	β	*P* value	β	*P* value
Age	-0.072	0.25	-0.061	0.35
Sex (men vs. women)	-0.011	0.86	-0.001	0.99
HOMAir	0.827	<0.001	0.773	<0.001
Glucose tolerance category				
IFG vs. NFG	-0.418	<0.001	-0.416	<0.001
T2DM vs. NFG	-0.726	<0.001	-0.744	<0.001
HDL anti-oxidative capacity	-0.051	0.56		
Interactions				
IFG x HDL anti-oxidative capacity	0.038	0.63		
T2DM x HDL anti-oxidative capacity	0.251	<0.001		
Cellular cholesterol efflux			0.014	0.87
Interactions				
IFG x cellular cholesterol efflux			0.003	0.97
T2DM x cellular cholesterol efflux			0.149	0.042

β: standardized regression coefficient. Interactions were calculated as the product term between IFG, T2DM, and HDL anti-oxidative capacity (model A), and between IFG, T2DM, and cellular cholesterol efflux to plasma (model B) using NFG as the reference category. HOMA-β and HOMAir were logarithmically transformed.

The replication group was comprised of 225 non-diabetic men (mean age 53 ± 12 years; BMI 26.6 ± 3.6 kg/m^2; plasma glucose 4.9 ± 0.6 mmol/l; insulin 8.3 (6.1–12.7) mU/l; HOMA-β 129 (87.3–203.8)%; HOMAir (2.30 (1.24–2.87) mU mol/l^2); total cholesterol 5.92 ± 1.13 mmol/l; HDL cholesterol 1.13 ± 0.35 mmol/l; apoA-I 1.29 ± 0.25 g/l). In these subjects, HDL cholesterol was correlated inversely with insulin (r = −0.201, *P* < 0.01), HOMA-β (r = −0.182, *P* < 0.05) and HOMAir (r = −0.239, *P* < 0.001), but not with plasma glucose (r = −0.091, *P* = 0.17). Essentially similar relationships were found with apoA-I (data not shown). In partial correlation analyses, the correlations of HOMA-β with HDL cholesterol and with apoA-I were not significant after controlling for HOMAir (r = −0.066, *P* = 0.34 and r = −0.035, *P* = 0.61).

Table 7.5. Multiple linear regression analyses demonstrating interactions between HbA1c and HDL anti-oxidative capacity (model A) and cellular cholesterol efflux to plasma (model B) on β-cell function (HOMA-β) in 95 subjects.

	Model A		**Model B**	
	β	***P* value**	**β**	***P* value**
Age	-0.242	0.008	-0.222	0.014
Sex (men vs. women)	-0.071	0.43	-0.035	0.69
HOMAir	0.585	<0.001	0.531	<0.001
HbA1c	-0.114	0.23	-0.203	0.023
HDL anti-oxidative capacity	0.058	0.50		
Cellular cholesterol efflux			0.087	0.30
Interaction				
HbA1c x HDL anti-oxidative capacity	0.222	0.015		
Interaction				
HbA1c x cellular cholesterol efflux			0.207	0.017

β: standardized regression coefficient. Interactions were calculated as the product terms between HbA1c and HDL anti-oxidative capacity (model A) and between HbA1c and cellular cholesterol efflux (model B). HOMA-β and HOMAir were logarithmically transformed.

Multiple linear regression analysis was carried out to discern whether the relationships of HOMA-β with HDL anti-oxidative capacity and cellular cholesterol efflux were different in T2DM subjects compared to NFG and IFG subjects. As demonstrated in Table 7.4, a positive interaction between glucose tolerance category and HDL anti-oxidative capacity on HOMA-β was observed after adjustment for age, sex, and HOMAir (model A; *P* < 0.001 for interaction term between T2DM and HDL anti-oxidative capacity with NFG as reference group). Likewise, there was also a positive interaction between glucose tolerance category and cellular cholesterol efflux on HOMA-β (model B; *P* = 0.042 for interaction term between T2DM and cellular cholesterol efflux to plasma with NFG as reference group). These interactions remained essentially similar after additional adjustment for either HDL cholesterol or apoA-I (model A: T2DM–HDL anti-oxidative capacity interaction: β = 0.249, *P* < 0.001 and β = 0.256, *P* < 0.001, respectively; model B: T2DM–cellular cholesterol efflux interaction: β = 0.153, *P* = 0.040 and β = 0.154, *P* = 0.039, respectively), and were not confounded by the use of anti-hypertensive drugs (model A: β = 0.252, *P* < 0.001; model B: β = 0.147, *P* = 0.046). Additionally, a positive interaction

between HbA1c (as continuous variable) and HDL anti-oxidative capacity on HOMA-β was observed (Table 7.5 model A, *P* = 0.015). There was also a positive interaction between HbA1c and cellular cholesterol efflux on HOMA-β (Table 7.5 model B, *P* = 0.017). These analyses thus demonstrated that the relationships of HOMA-β with HDL anti-oxidative capacity and with cellular cholesterol efflux to plasma were different in T2DM subjects compared to NFG subjects, and varied according to the degree of metabolic control, taking account of insulin sensitivity.

7.4 Discussion

To our knowledge this study demonstrates for the first time that in well-controlled T2DM subjects pancreatic β-cell function is related positively to pathophysiologically relevant functional properties of HDL, as represented by the capacity to inhibit LDL oxidation and the ability of plasma to stimulate cellular cholesterol efflux. No such relationships were observed in subjects with NFG and IFG subjects. Positive interactions between glucose tolerance category and both measures of HDL functionality on pancreatic β-cell function were observed, underscoring the notion that the relationship of β-cell function with HDL functionality was indeed different in T2DM compared to NFG. In addition, the HbA1c level also interacted positively with these HDL functionality measures on pancreatic β-cell function. Of further relevance, neither HDL anti-oxidative capacity nor cellular cholesterol efflux to plasma was significantly different in T2DM compared to NFG and IFG subjects. Overall, the present results conceivably indicate that better HDL functional properties may contribute to maintenance of β-cell function in subjects with well-controlled T2DM. It seems unlikely from the current observations that defective HDL functionality provides a major contributing mechanism that is primarily responsible for the deterioration of β-cell function that occurs during the development of T2DM.

In this study pancreatic β-cell function and insulin sensitivity were assessed using homeostasis model assessment equations.[22] As expected, relative β-cell function and insulin sensitivity were both impaired in T2DM subjects compared to NFG subjects.[21, 25] Moreover, the HOMA-β/HOMAir ratio was progressively decreased with deteriorating glucose tolerance, supporting the concept that glucose tolerance will deteriorate when the adaptive β-cell response becomes insufficient.[21] In the 3 glucose tolerance categories and in all participants combined there was a close correlation between HOMA-β and HOMAir, which should be explained in view of the intricate interdependence between HOMA-β and HOMAir, as reflected by the mathematical equations used to calculate β-cell function and insulin sensitivity.[22] This makes it necessary to take account for HOMAir when evaluating relationships of HDL functionality measures and HDL cholesterol with HOMA-β. Indeed, we replicated inverse univariate correlations of HOMA-β with HDL cholesterol which were no longer significant after controlling for HOMAir in another cohort of non-diabetic men. Presumed effects of HDL on insulin sensitivity [5, 14] and vice versa inverse relationships of HDL cholesterol with insulin sensitivity (among others ref. [3] and confirmed in the present study) provide additional arguments to correct for HOMAir in establishing relationships of HDL functionality measures with HOMA-β. In T2DM subjects, the positive relationships of HDL anti-oxidative capacity, as well as with cellular cholesterol efflux to plasma with HOMA-β remained present after controlling for HOMAir. In NFG and IFG subjects, HDL anti-oxidative capacity and cellular cholesterol efflux to

plasma were not related to HOMA-β after adjustment for HOMAir. Neither measure of HDL functionality was significantly correlated with HDL cholesterol and plasma apoA-I, whereas HDL cholesterol and apoA-I did not independently contribute to HOMA-β in multivariate linear regression models. Taken together, these findings support the concept that HDL functionality measures may be advantageous over mere HDL cholesterol and apoA-I mass measurements in establishing relationships of HDL with pancreatic β-cell function.

We employed HDL anti-oxidative capacity and cellular cholesterol efflux as read outs for HDL functionality. In our report, the anti-oxidative function of HDL was not different in T2DM compared to NFG and IFG subjects using an assay system that is standardized for the amount of HDL cholesterol. Using a comparable type of assay impaired anti-oxidative function was attributed to specific defects in the HDL_3 fraction from T2DM patients with much more pronounced hyperglycemia and dyslipidemia compared to the presently studied T2DM subjects.[26] Differences in patient selection and in methodology (anti-oxidative functionality in whole HDL vs. HDL subfractions) may at least in part explain the apparent discrepancy. In accord with an inhibiting effect of the degree of hyperglycemia on HDL anti-oxidative function,[27] we observed an inverse relation of HDL anti-oxidative capacity with fasting plasma glucose. Of further note, in T2DM subjects an inverse correlation of skin autofluorescence, as a proxy of tissue advanced glycation end-product accumulation, with the HDL anti-oxidative capacity was documented recently, which supports the potential clinical relevance of HDL's anti-oxidative properties as assessed with this assay system.[18] In the current study, human cultured fibroblasts obtained from a single normolipidemic donor were used to determine the ability of each individual's plasma to stimulate cholesterol efflux.[19] These cells were cholesterol loaded in order to induce ABCA1 expression.[19] Although the relative contribution of the various processes involved in cholesterol efflux out of different cell types is still uncertain,[12, 28, 29] evidence has accumulated that the ABCA1 pathway represents a key cholesterol transport system for β-cells in rodents [8-10] and humans.[13] In this context it is relevant that cholesterol efflux out of fibroblasts using the same assay with whole diluted plasma as cholesterol acceptor in the medium is correlated positively with plasma levels of lipid-poor preβ-HDL particles,[30] concurring with *in vitro* observations that these particles represent initial acceptors of cell-derived cholesterol via the ABCA1 pathway.[12, 29] Although we cannot completely rule out some contribution to the efflux rates measured of the presence of LDL and VLDL in the efflux medium, confounding attributable to the reuptake of newly effluxed labelled cholesterol was excluded under the currently used assay conditions.

Several other methodological issues need to be considered. First, we excluded T2DM subjects using glucose-lowering drugs. This was done to avoid confounding due to actions of sulfonylurea and metformin on insulin secretion and insulin sensitivity.[30] We also excluded subjects using cholesterol-lowering medications in order to avoid drug-induced effects on HDL metabolism and HDL functionality.[3] Consequent to these exclusion criteria, the participating diabetic subjects were in general metabolically well regulated and their plasma lipid levels were remarkably similar compared to NFG and IFG subjects. Thus, the positive relationship of HDL functionality measures with pancreatic β-cell function in T2DM subjects may be different in the context of more pronounced hyperglycemia and dyslipidemia. Second, the number of T2DM subjects enrolled in this study was rather small. However, our finding that the relation of HDL functionality measures with pancreatic

β-cell function varied between T2DM compared to NFG and IFG subjects was extended by additional analyses using HbA1c levels as measure of metabolic (dys)regulation. Third, given the cross-sectional design of our study, no conclusion is allowed as to whether HDL functionality measures predict deterioration of β-cell function over time. Finally, we suggest that the positive effects of HDL functionality on HOMA-β in T2DM subjects, as a relative measure of β-cell function warrant further exploration employing dynamic tests of insulin secretion.

In conclusion, pancreatic β-cell function relates positively to indices of HDL functionality in well-controlled T2DM, but not in NFG and IFG. Better HDL functionality may, therefore, contribute to maintenance of β-cell function in subjects with established T2DM. Assessment of HDL functionality appears to be advantageous over HDL cholesterol measurement in establishing novel roles of HDL in the pathophysiology of glucose homeostasis.

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